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Supramolecular pentapeptide-based fullerene nanofibers: effect of molecular chirality†

Alberto Insuasty,[a] Carmen Atienza,[a] Juan Luis López[a] and Nazario Martín*[ab]

The supramolecular organization of new fullerene derivatives endowed with peptides as biomolecular templates affords ordered nanofibers of several micrometres length based on hydrogen bonds and π–π interactions.

Control over the formation of nanostructures by the self-assembly of electroactive molecules is an important issue in the search for applications such as photovoltaics and organic electronics, where morphology plays a critical role.[1] In this regard, the self-organization of electroactive fullerenes has attracted considerable attention because of their singular shape, high electron affinity and capacity for charge transport, which make them important 3D building blocks in supramolecular chemistry.[2] Furthermore, the self-organization of fullerenes has resulted in remarkable charge carrier mobilities within highly ordered domains, which have found applications in organic electronic devices such as field effect transistors (FETs) and organic solar cells (OSCs), to name a few.[3]

The assembly of fullerene derivatives has given rise to a variety of nanostructures with different sizes and shapes. Some of these are based on suitably functionalized fullerenes endowed with large side chains to form amphiphilic systems and others consist of co-polymers and co-assemblies giving rise to ordered electroactive domains.[4] However, the use of biomolecular templates as an alternative concept for organizing fullerenes has been barely explored.[5] In this regard, biomolecular templates are attractive target molecules to form highly complex supramolecular structures due to their well-defined chemical, physical and structural properties. In particular, peptide-based tubular systems or DNA have been successfully used to arrange photo- and electro-active carbon nanostructures such as fullerenes or single wall carbon nanotubes (SWCNT). As a matter of fact, the resulting hybrids exhibit outstanding redox and optoelectronic properties.[6]

Among the different biomolecular templates, peptides have frequently been used in supramolecular chemistry. Thus, short peptide units allow access to a variety of morphologies at the nano- and meso-scale and, therefore, make these units an important tool to be considered when different types of nanostructures are desirable.[7,8] In addition, supramolecular chemistry based on peptides has found applications in biomedical areas with promising results in a variety of research fields.[9]

In this context, we now describe the straightforward formation of C_{60}-nanofibers using different peptide sequences as biomolecular templates capable of self-assembly through multiple hydrogen-bonding interactions, resulting in stable supramolecular architectures. For this purpose, we have synthesized a new series of fullerene derivatives (**1–3**) based on their covalent linkage to pentapeptide units (Fig. 1).

It is important to remark that, in this molecular design, we have considered two main issues: (i) the presence of the peptide

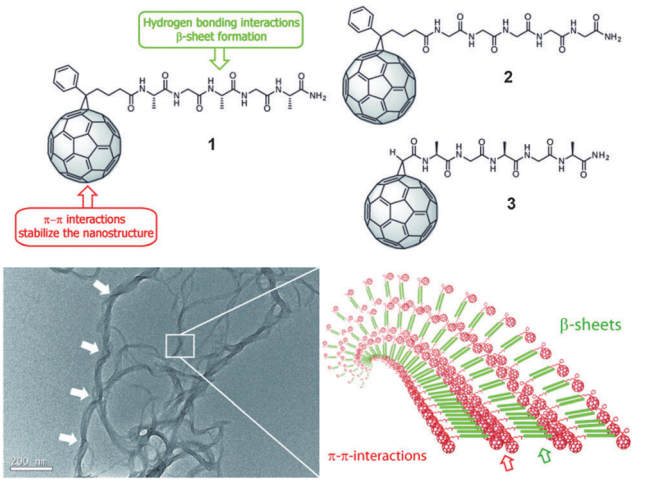

Fig. 1 Chemical structures of the novel C_{60}-pentapeptide compounds **1–3** (Top). TEM image of compound **1** in TCE : DMSO (10%) and a schematic representation of its assembly showing the β-sheets and π–π interactions between the C_{60} units, which stabilize the nanostructure formed (Bottom).

[a] *Departamento de Química Orgánica, Facultad de C. C. Químicas, Universidad Complutense de Madrid, 28040 Madrid, Spain. E-mail: nazmar@ucm.es; Fax: +34 91-394-4227*
[b] *IMDEA-nanociencia, 28049 Madrid, Spain. Fax: +34 91-394-4103*

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ensures the formation of the β-sheet as a secondary structure, which stabilizes the nanostructure formed and (ii) the use of non-planar 3D units such as fullerene derivatives allows the stabilization through intermolecular π–π interactions in the nanostructures obtained. Furthermore, fullerenes are in turn excellent n-type organic semiconductors of interest for a variety of purposes.

Compounds **1** and **2** are based on a [6,6]-phenyl-C_{61}-butyric acid methyl ester derivative (PCBM) functionalized with a chiral pentapeptide (L-Ala–Gly–L-Ala–Gly–L-Ala–NH_2) and an achiral (Gly–$(Gly)_3$–Gly–NH_2) one, respectively. Compound **3** is, however, a [6,6]-carboxymethano[60]fullerene derivative comprising the same chiral pentapeptide as compound **1**. The aim of using these [60]fullerene derivatives with different chemical diversity is to compare the morphological changes stemming from the peptide's chirality (compounds **1** and **2**) as well as the effect of the proximity of the chiral pentapeptide to the C_{60} surface (compounds **1** and **3**).

Pentapeptides L-Ala–Gly-L-Ala–Gly–L-Ala–NH_2 and Gly–$(Gly)_3$–Gly–NH_2 were synthesized by fluorenylmethoxycarbonyl (Fmoc) solid-phase synthesis on a 4-methylbenzhydrylamine (MBHA) resin. Removal of the Fmoc group and subsequent cleavage from the resin afforded the desired peptides, bearing amine and amide groups at the N- and C- termini, respectively. Finally, an amidation reaction between the pentapeptides and PCBM carboxylic acid or [6,6]-carboxymethano[60]fullerene afforded **1–3** in good yields. For synthetic details, see the ESI.†

Compounds **1–3** showed only good solubility in dimethyl sulfoxide (DMSO) and partial solubility in 1,1,2,2-tetrachloroethane (TCE). Therefore, we used a mixture of TCE and 10% of DMSO (v/v), conditions in which their self-assembly is efficiently achieved. The supramolecular structures resulting from the self-assembly of **1–3** have been investigated through spectroscopic, chiroptical and microscopic techniques.

Transmission electron microscopy (TEM) imaging obtained by drop casting solutions of **1–3** in the mixture TCE : DMSO (10% v/v) showed the formation of nanofibers that were several micrometers in length in all cases, as shown in Fig. 1 and 2. It is possible to appreciate the differences between compounds **1–3**. In the case of compound **1**, Fig. 1 clearly shows how the fibers are twisted at some points along the fiber (see the white arrows in Fig. 1 and Fig. S3, ESI†), while in the case of compound **2** straight fibers are obtained instead (Fig. 2A).

At this point, it is important to remark that it is very difficult to correlate chirality with the nanostructure obtained. As it has previously been described in the literature, two important aspects should be considered: (i) chiral peptides endowed with planar units can lead to twisted arrangements at the nano- and meso-scale.[10] However, this is not a general trend and non-twisted nanostructures can be formed as well; and (ii) achiral molecules could give rise to twisted arrangements.[11]

In this regard, considering the non-planar chemical structures of compounds **1** and **2** (Fig. 1 and 2), we hypothesize that this nanostructural difference could be accounted for by the role of peptide's chirality, which is expressed on the nano-scale in the supramolecular structure. However, in the case of compound **3**, the fibers formed seemed to be straighter, shorter and more aggregated than those obtained from compounds **1** and **2**. We believe that these features might be presumably attributed to the suppression of the alkyl chain by three carbon atoms of the PCBM, making **3** a more rigid system when compared to **1** and **2**. This more rigid system in **3** allows better packing between the β-sheets strands through π–π interactions between the C_{60} molecules, whereas in **1** and **2**, these molecules have a higher degree of freedom with respect to the β-sheet owed to the presence of the alkyl chain. With the aim of obtaining a better insight of the fine structure of the nanofibers, high-resolution transmission electron microscopy (HRTEM) images were obtained. Unfortunately, after several attempts, no clear pattern could be observed (see Fig. S4 in the ESI†).

Complementary studies performed by atomic force microscopy (AFM) showed very similar trends to those observed in TEM regarding the morphological features, as shown in Fig. 3. The formation of long fibers and agglomerated structures were observed in all cases. Inspection of the heights over the nanofibers revealed that the smaller ones correspond to the expected heights for one (~3.4 nm), two (~7 nm) or three (~10 nm) strands of β-sheets, as shown in the height profiles in Fig. 3D and E.

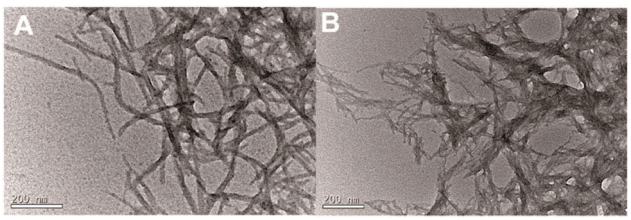

Fig. 2 TEM images of compounds **2** (A) and **3** (B) in TCE : DMSO (10%).

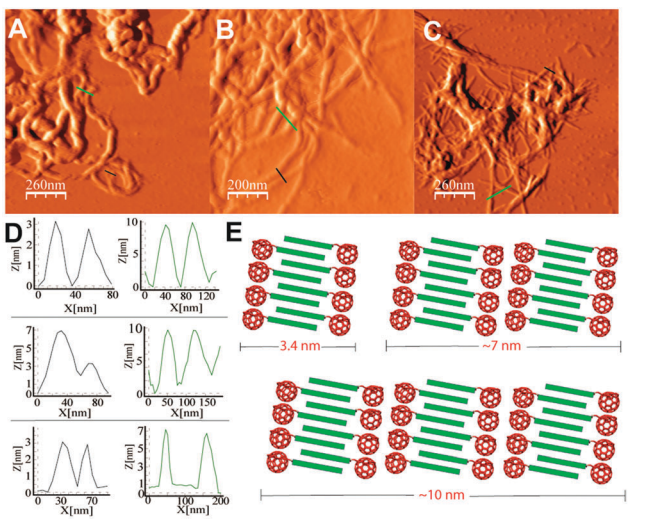

Fig. 3 AFM images obtained by drop casting over freshly cleaved mica. (A), (B) and (C) correspond to the nanofibers obtained from compounds **1**, **2** and **3**, respectively. (D) Height profiles corresponding to the lines marked in (A)–(C). The upper, middle and lower part correspond to (A), (B) and (C), respectively. (E) Schematic representation accounts for the height profiles observed in AFM corresponding to one, two and three strands of the β-sheets.

The agglomerated structures should consist of several packed strands, which are further stabilized by π–π interactions between the C_{60} units.

Fourier transform infrared (FTIR) spectroscopy measurements were performed in order to confirm the formation of β-sheets as the secondary structure of **1–3**. A strong amide I ($\nu_{C=O}$) band at 1630 cm^{-1} together with a weak shoulder around 1690 cm^{-1} were observed, which is in agreement with the existence of intermolecular β-sheets. These features, as it has previously been reported, correspond to peptide units interacting in an antiparallel mode.[7,10b,12] In some cases, the band at 1650 cm^{-1} was barely observed, which indicates the existence of random aggregates (Fig. S1 in the ESI†).

The optical properties of **1–3** were measured in DMSO and TCE : DMSO (10% v/v). In DMSO, the UV-vis spectra of **1–3** showed the typical fullerene bands at 330 and 430 nm, the latter associated to the saturation of a C–C double bond in the fullerene monoadducts.[13] However, the UV-vis spectra of **1–3** in a mixture of TCE : DMSO revealed a depletion of the band at 330 nm together with an increase in absorbance around ~450 nm (see the red arrows in Fig. 4A–C). This trend has also been observed in fullerene aggregates,[14] films[15] or C_{60} molecules packed within constrained channels[16] and has been attributed to interactions between the C_{60} units,[17] thus being indicative of the aggregation of the fullerene moieties. In the case of compound **3**, the broadening is more significant than the other compounds, losing the feature band at 430 nm. This feature might be in accordance with higher packing of C_{60} molecules in compound **3** when compared to **1** and **2** owing to the higher rigidity of the system.

The self-assembly of **1–3** was also monitored by circular dichroism (CD) in DMSO and a mixture of TCE : DMSO. The CD spectra in DMSO showed no optical activity for compounds **1** and **2** and a weak dichroic signal for compound **3** as shown in Fig. 4D–F (black lines). Herein, it is important to remark that despite the fact compounds **1** and **3** are covalently bonded to a chiral peptide, the dichroic signal was solely observed for compound **3**. This fact could be attributed to the proximity effect of the first chiral center (Ala) when compared to molecule **1**, in which its chiral center is further from the π-cloud of the [60]fullerene.[18] These low CD responses could be in agreement with the presence of disaggregated compounds. However, the CD studies for **1** and **3** in TCE : DMSO exhibited a notable negative Cotton effect in the range of the fullerene absorptions. These experimental findings resemble that observed for a related system when the molecule is assembled.[7] Thus, the results support the existence of supramolecular aggregates in which a transfer of chirality occurs from the peptide to the fullerene. It is worth to mention that in the case of compound **3**, the response in the CD measurements is higher when compared to compound **1**. This outcome could be accounted for by the proximity effect of the peptide chain to the C_{60} sphere when compared to compound **1**. As a matter of fact, in the case of compound **2**, the lack of chirality in the peptide chain does not trigger any response in the CD measurements in any case, neither disaggregated (DMSO) nor assembled (TCE : DMSO).

Fluorescence studies for compounds **1–3** were performed in DMSO and a mixture of TCE : DMSO (Fig. S2 in the ESI†). The intensity of the [60]fullerene fluorescence with a maxima at ~709 nm decreased and shifted slightly in the mixture of TCE : DMSO when compared to that obtained in DMSO. This slight fluorescence quenching can be presumably attributed to the fact that florescence is more deactivated when C_{60} molecules are closer, *i.e.*, in the mixture of TCE : DMSO. However, due to the fact that fluorescence changes are very weak in all cases, any clear conclusion cannot be derived.

In summary, the present study shows that pentapeptide biomolecular templates serve as effective tools to organize fullerene molecules. The nanofibers are stabilized through the β-sheets along with π–π interactions between the C_{60} molecules. Moreover, a study on nanofiber's morphology based on its molecular features such as peptide's chirality and the peptide's distance to the C_{60} unit has been performed using different techniques (UV-vis, fluorescence, FTIR, CD, TEM and AFM). Interestingly, CD studies in TCE : DMSO show a strong negative Cotton effect in the range of fullerene absorptions, which reveals an efficient chirality transfer from the chiral peptide unit to the C_{60} moiety. Furthermore, this effect depends of the distance existing between them. The highly ordered electroactive materials formed as well as the ease in the experimental procedure used show that the use of biomolecular templates is a very attractive approach in the search for supramolecularly organized fullerenes. Since fullerene assemblies are required for a variety of applications in different devices, we feel that our results pave the way to the supramolecular organization of electroactive 3D fullerenes based in bio-inspired H-bonds and π–π interactions.

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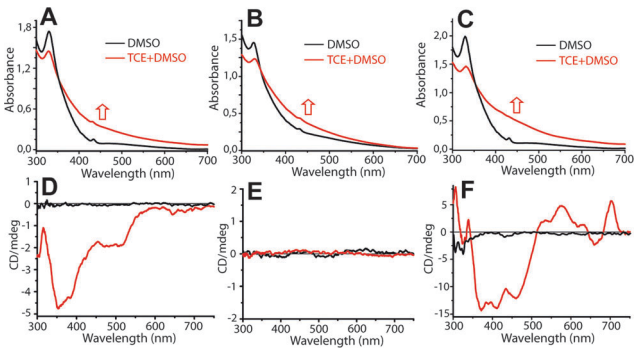

Fig. 4 UV-vis spectra in DMSO (black lines) and a mixture of TCE : DMSO (10% v/v, red lines) for compounds **1** (A), **2** (B) and **3** (C). The red arrows show the increase in absorbance at around 450 nm. Circular dichroism spectra in DMSO (black lines) and a mixture of TCE : DMSO (10% v/v, red lines) for compounds **1** (D), **2** (E) and **3** (F).

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